

AN ANALYTICAL INVESTIGATION

OF

GARDEN SAGE

(*SALVIA OFFICINALIS*, LINNÉ)

A DISSERTATION

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY IN THE FACULTY OF
PURE SCIENCE OF COLUMBIA UNIVERSITY

By

LEON LAIZER WATTERS, B.S., A.M.

NEW YORK

1901

AN ANALYTICAL INVESTIGATION

OF

GARDEN SAGE

(SALVIA OFFICINALIS, LINNÉ)

A DISSERTATION

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY IN THE FACULTY OF
PURE SCIENCE OF COLUMBIA UNIVERSITY



By

LEON LAIZER WATTERS, B.S., A.M.

NEW YORK

1901

PRESS OF
THE NEW ERA PRINTING COMPANY,
LANCASTER, PA.

ACKNOWLEDGMENT.

The author desires to extend his thanks to Professor Edmund H. Miller for having provided the facilities which made this work possible, and to Dr. H. C. Sherman for numerous suggestions during its course.

L. L. W.

Analytical Laboratories, Havemeyer Hall, May, 1901.



Digitized by the Internet Archive
in 2026

HISTORY.

The use of sage as a condiment, and as a medicine dates back many centuries. It appears to have been used as a medicinal herb at the time of the Romans, and was one of the plants recommended by Charlemagne for cultivation. Arnoldus Villanovus (1235–1312), was acquainted with the distillation of the oil¹ which was further described by Raymundus Lullus.² The oil is first mentioned in the “Price of Ordinances of Worms” of 1582, and of Frankfurt of 1587, and it is included in the 1589 edition of the *Dispensatorium Noricum*. The oil was further described by Begnini in 1688, and the yield of oil from the leaves was determined by Wedel in 1715 and by Cartheuser about 1732.³ Considerable attention has since been paid to the oil. In 1720 Geoffroy observed a stearoptene which crystallized from the oil, and which he termed sage camphor.⁴ The same substance was again observed and described by Arezula in 1789.⁵ The oil has been further investigated by Herberger in 1829,⁶ and by Rochleder in 1841.⁷ Later, it was more closely studied by Tilden,⁸ Muir and Sugiura,⁹ and by Wallach.¹⁰

The writer has been able to find but one authenticated record of past investigations upon the sage plant itself in form of an analysis by Ilisch and Riga made almost a hundred years ago,¹¹ which appears to be the basis upon which the statements of the composition of sage occurring in works of reference are made. They concluded that

¹ Opera omnia, Lib. de vinis, p. 589.

² Experimenta nova, vol. 5, fol. 829.

³ Elementa Chymiae, vol. 2, p. 87.—Gildemeister & Hoffman, “Die aetherische Oele,” p. 612.

⁴ Resultado de las experiencias hechas sobre el alcanfor de Murcia non licencia. Segovia, 1789, p. 8.

⁵ Mem. de l'Acad. roy. des sciences de Paris, 1721, p. 163.

⁶ Buchner's Repert f. d. Pharm., 34, p. 131.

⁷ Liebig's Annalen, 44, p. 4.

⁸ Jour. Chem. Soc., 1877, 1, p. 554.

⁹ Phil. Mag. and Jour. of Sciences, vol. 4, p. 336.—Pharm. Jour., III, 8, pp. 191, 994.—Jour. Chem. Soc., 1877, 11, p. 548.—Chem. News, 37, p. 211; Jour. Chem. Soc., 33, p. 292.—Jour. Chem. Soc., 37, p. 678.—Chem. News, 41, p. 223.

¹⁰ Liebig's Annalen, 252, p. 103; 227, p. 289.

¹¹ Trommsdorff's Journal der Pharmacie, Vol. 20, part II, pp. 7–16. 1810.

sage contained in addition to the usual plant constituents, a green resin, a gummy substance and an essential oil. Free malic acid was also claimed as a constituent of sage, though the evidence upon which the claim is made does not appear, beyond minor physical indications. Ilisch and Riga reported no tannin, though various botanical text-books¹ include an iron-greening tannin among the constituents of sage. An uncredited statement appears in the National Dispensatory² to the effect that Hirsch did not succeed in obtaining tannin. It has been found impossible either to trace or to verify this reference.

GENERAL METHODS OF ANALYSIS.

In the work which follows, a determination of the various extracts was first made as nearly as practicable according to the usual methods of plant analysis, and later the more important constituents were determined by special methods. One series of determinations was made upon extracts obtained by hot extraction; a second upon extracts obtained by cold extraction; in both cases the amounts of the extracts were obtained directly by weighing the residues left after evaporating the solvent. A third series of determinations was made by hot extraction, in which the amounts of the extracts were determined indirectly by difference in the weights of the material before and after extraction. At least one check was run in each case.

Hot Extraction: For this purpose a somewhat modified Soxhlet extractor was used, in which the usual inner tube was replaced by a simple glass cylinder, having a constriction near the lower end over which a muslin filter was tied. This permitted of more rapid extraction. The extractions were usually continued for about 30 hours, when the solution was filtered to remove suspended matter and made up to an even bulk with the addition of as little of the solvent as possible.

Cold Extraction: The method used was practically that outlined by Dragendorff.³ The macerations were continued for a week, except in the cases of extraction by water, soda, and acid, where 24

¹ Bentley and Trimens: *Medicinal Plants*, p. 206.—Wittstein: *Handwörterbuch der Pharmakognosie des Pflanzenreich's*, Breslau 1882, p. 713.

² Ed., pp. 1405.

³ "Pflanzenanalyse," Göttingen 1881.

hours sufficed. The material was usually macerated with ten times as many cubic centimeters of solvent as there were grams of the material originally used. The whole was frequently shaken during maceration, and at the end, the clear liquid was decanted through a dry filter, the residue well washed with fresh solvent, and the filtrate and washings made up to an even volume.

Estimation by Difference: The experiment was tried of estimating the various extracts indirectly by the difference in weight of the material before and after extraction by each solvent, as a check upon the usual method in which the extracts are estimated directly by evaporating the solvent after extraction, and weighing the residue thus obtained. For this purpose it was necessary, that the residue of the original sample be brought to the same condition of dryness after each extraction. Drying in a vacuum over concentrated sulphuric acid was tried but it was finally decided to dry to constant weight at 100° after each extraction. Under these conditions results were obtained which compared favorably with the results given by direct estimation. The extractions were carried on in Soxhlet apparatus using an ordinary inner tube with a muslin filter. A circular perforated porcelain disc served to prevent the drug from clogging the filter. After each extraction the residue was drained as completely as possible, and transferred to a tared watch glass by tapping the tube. After a few minutes further drying in the air, the muslin filter was removed and all adhering particles of the material brushed out into the watch glass, which, with contents was dried to constant weight in a water-oven, and weighed.

Evaporations of the solvent were made in dishes such as are commonly used in bacteriology. These had vertical sides, and ground edges, and were fitted with ground-glass covers. In the case of the petroleum ether extract it was found convenient to use thin lead capsules which prevented creeping. Evaporations were conducted in the air at the ordinary temperature; no advantage was found in evaporating the solvent under reduced pressure or in a current of dry air. As considerable variation was noticed in the weights of some of the extracts air-dried, all weighings were first made upon the air-dried extracts which were then further dried, according to the nature of each extract, and reweighed.

The order of solvents used was as follows: (1) Petroleum ether

(B. P., 45°C.); (2) ethyl ether (dried over sodium); (3) absolute alcohol; (4) water; (5) 0.15-per-cent. soda solution; (6) 1-per-cent. hydrochloric acid. The same order of solvents was used also in the Soxhlet extractions, up to the point of extraction with water, where extraction in the Soxhlet apparatus was found to be impracticable, and extractions by subsequent solvents were made by cold maceration.

The determinations which follow are not upon the same residues throughout, as in some cases several determinations had to be made before a satisfactory result was obtained.

ANALYSIS PROPER.

Sample: The sample consisted of the leaves and stems of what is known in the trade as the "large-leaf" variety of sage obtained from a reliable source. It was cultivated near Frankfurt a/M, Germany, and picked in June, 1900, or about six months previous to the beginning of this work. It fulfilled the requirements of the Pharmacopœia for *Salvia officinalis*, Linné, and was such as would be dispensed as a good quality of sage. For analysis about 2 kg. was first freed from dirt and any admixture of foreign substances, picked from twigs, pounded in an iron mortar without further drying and finally ground and powdered in a stoneware mortar till it passed easily through a sieve of 40 meshes to the inch, and preserved in glass-stoppered bottles.

Moisture: 2 gms. of sample (A) in a shallow platinum dish was heated in a water-oven for three hours; two similar portions (B and C) spread out on watch glasses were heated in an air-bath at 100° C. for three hours.

Weight sample before.....	2.000	2.000	2.000
Weight sample after.....	1.826	1.827	1.833
Loss at 100°.....	0.174	0.173	0.167
Per cent. loss at 100°.....	8.70	8.65	8.35
Average per cent.....	8.54		

For a discussion of the various methods for determining moisture in the presence of volatile oil see pp. 22-23.

Ash: Two portions of 3 gms. each were incinerated to a grayish-white ash, the ash weighed, and its solubility in water, dilute hydrochloric acid and 20-per-cent. sodium hydroxide determined.

	A	B
Weight sample	3.000	3.000
Weight ash.....	0.331	0.324
Per cent. ash.....	11.03	10.80
Average per cent.....	10.91	
Soluble in water	3.50	per cent.
Soluble in dilute hydrochloric acid.....	4.34	"
Soluble in 20-per-cent. sodium hyroxide.....	1.73	"
Insoluble residue.....	1.23	"
Total	10.80	"

EXTRACTION WITH PETROLEUM ETHER.

Extraction Hot: (1) Two portions of sage were extracted in Soxhlet apparatus as described for 30 hours.¹

The solution of 20 gms. (A) was made up to 300 c.c., of which 50 c.c. was evaporated to dryness in the air and the extract weighed. The whole of the solution from 10 gms. (B) was similarly evaporated to dryness, and the residue weighed.

	A	B
Weight sample.....	20.000	10.000
Weight extract.....	1.189	0.553
Per cent. petroleum-ether extract.....	5.94	5.53

Average petroleum-ether extract, air-dried = 5.74 per cent.

(2) The percentage for total extract as above obtained was high as compared with the determination by difference made later, due to non-volatilization of some of the solvent by drying in the air. This was again shown in the determination which follows, where weighing the air-dried residues gave abnormally high results, while after a few minutes' careful heating results were obtained which agree quite closely with those obtained by difference. Five grams of sample was extracted with pretroleum ether as above, the solution made up to 100 c.c., and two portions of 20 c.c. each evaporated dry in the air. The residues were weighed air-dried, then heated at 60°–70° C. for ten minutes and again weighed.

¹To determine if all soluble matter had been removed, the marc thus extracted was reextracted for 3 hours with fresh petroleum ether. Upon evaporating the solvent, a residue of but 0.0015 gm. was obtained, showing the extraction to have been practically complete.

	C	D
Weight residue, air-dried	0.1287	0.1264
Per cent. residue, air-dried.....	12.87	12.64
Weight residue after heating.....	0.0449	0.0444
Per cent. residue after heating.....	4.49	4.44
Average per cent.....	4.47	

Extraction Cold: 50 gms. sample was macerated for a week with 400 c.c. petroleum ether. The clear solution was then decanted through a dry filter, the residue washed with fresh solvent, and the solution made up to 500 c.c. Five portions of 10 c.c. each were evaporated dry in the air and weighed for total extract.

	E	F	G	H	I
Weight residue	0.0617	0.0564	0.0568	0.0598	0.0638
Per cent. "	6.17	5.64	5.68	5.98	6.38
Average 5.97 per cent.					

A sixth portion (J) of 10 c.c. was evaporated dry in the air and then heated 10 minutes at 60–70° C.

Weight residue.....	0.0493
Per cent. "	4.93

Estimation by Difference: 3 gms. sample was first dried at 100° for 2½ hours in order that the first weighing could be made under the same conditions as those following each extraction, where the residues were similarly heated. The residue weighing 2.7325 gms. was then extracted as already described, and then transferred from the tube to tared glasses and dried to constant weight:

Weight before extraction.....	2.7325
Weight after extraction	2.5828
Loss.....	0.1497
Per cent. petroleum-ether extract by difference....	4.99

These results show that in this particular case at least, the weight of the air-dried residue cannot be made use of in calculating the total extract, such varying from 5.53 (B) to 12.87 per cent. (C) according to the conditions of the evaporation. This variation is due to small quantities of the solvent, which in the case of an extract of the consistency of that of sage are not easily removed by simple drying in the air as is usual. Heating the air-dried residues for a few minutes at a temperature somewhat above the boiling point of petroleum ether gave results which were fairly constant, thus:

(C) 4.49, (D) 4.44, (J) 4.93, per cent.

these results correspond tolerably well with that given by difference viz., 4.99 per cent. (See page 6.)

The petroleum-ether solution was brownish green in color, due to a little chlorophyll brought into solution by the oil present. It consisted essentially of a mixture of wax and fixed and volatile oils.

Volatile Oil: The volatile oil determined by the method given on page 28 gave 0.96 per cent.

Fixed Oil, Wax., etc.: 300 c.c. of the solution from 50 gms. of sage was freed from solvent in the air, and then heated at 110° C. for four hours to drive off the volatile oil. The residue was a dark green tenacious mass, without odor or taste. It was not easily soluble in petroleum ether, but dissolved readily in alcohol, which solution left a greasy spot on paper.

The residue of wax and fixed oil after heating at 110° amounted to 3.51 per cent.

EXTRACTION WITH ETHYL ETHER.

Extraction Hot: The residues from 20 gms. (A) and 10 gms. (B) after extraction with petroleum ether were air-dried and similarly extracted with ethyl ether for 30 hours. Here also the air-dried extracts gave results that were too high, and the residues were heated for a few minutes in a water-oven to drive off the last traces of the solvent.

	A	B
Weight residue.....	0.8135	0.4295
Per cent. residue.....	4.07	4.29
Average ethyl ether extract by hot extraction = 4.18 per cent.		

Extraction Cold: The residue of 50 gms. after extraction by petroleum ether was similarly extracted with ethyl ether, and the solution made up to 500 c.c. 300 c.c. was evaporated in a tared dish.

Weight residue air-dried.....	1.5699
Per cent. residue air-dried.....	5.233
Weight residue after heating 10 minutes at 100°C.....	1.4804
Per cent.....	4.94

As this result was somewhat higher than that given by hot extraction, a second determination was made using smaller amounts of the solution. Five portions of 10 c.c. each were evaporated dry in the air, weighed air-dried, then heated in a water-oven for an hour and again weighed.

	A	B	C	D	E
Weight residue air-dried	0.0531	0.0522	0.0527	0.0519	0.0517
Per cent. " " "	5.31	5.22	5.27	5.19	5.17
Weight residue dried at 100°....	0.0500	0.0490	0.0503	0.0492	0.0483
Per cent. " " " "	5.00	4.90	5.03	4.92	4.83
Average	4.94 per cent.				

Estimation by Difference: The residue from extraction by petroleum ether after being brought to constant weight at 100° was similarly extracted with ether, the residue again dried at 100° and the loss noted.

Weight residue before.....	2.5828
" " after	2.4573
Ether extract by difference	0.1255
Per cent. " " "	4.18

The ether solution was brownish green in color, and on evaporation left a light green amorphous residue. This proved to consist almost entirely of a green resin which was isolated as follows. The solution from 10 gms. of sage was freed from solvent by evaporation in the air and the residue transferred to a tared platinum Gooch crucible, which was then dried at 100° and weighed. It was then treated repeatedly with water, again dried and weighed.

Amount soluble in water.....	0.0725 gm.
Per cent. " " "	0.73

The residue was then treated with absolute alcohol in which it practically all dissolved. Soluble in alcohol 3.57 per cent.

The water solution gave no indications of the presence of hæmatoxylin, benzoic, salicylic or gallic acids. Evaporated to dryness in a platinum dish it left a very slight residue having a sharp taste.

The alcoholic solution was evaporated to dryness, and heated for a few minutes in a water-oven. There remained a fine pea-green residue which easily crumbled to an amorphous powder, which exhibited the properties of an acid resin. (See page 14.)

EXTRACTION WITH ALCOHOL.

Extraction with alcohol gave results that varied considerably with the conditions of the extraction, and the subsequent drying of the residues, due in part to actual differences in the amounts extracted, and in part to the difficulty of bringing the extracts to constant

weight without change in their composition. A number of extractions were made, both hot and cold. The results given are those last made which agree quite well. It was found impossible to completely extract the material by cold maceration.

Extraction Hot: The residues after extraction with ether, were air-dried, and similarly extracted with absolute alcohol. In the case of one portion (residue of 10 gms.), there was some decomposition of the extract due to over-heating. The solution from the 20-gm. portion was made up to 350 c.c. and one-fifth removed and evaporated to dryness in a platinum dish on a water-bath for total extract. The residue was then heated in a water-oven till two consecutive weighings showed a difference of less than 3 mgms.

Weight residue air-dried.....	0.6405
“ “ after six hours' heating.....	0.5265

The dish was then ignited and the extract burned to an ash which was deducted.

Weight ash.....	0.0112
“ alcoholic extract.....	0.5153
Per cent. “ “	12.88

The extract thus obtained resembled in all respects that obtained by cold maceration.

Extraction Cold: In order to make the extraction as complete as possible, the residue of 50 gms. of sage after extraction with ether, was air-dried, and macerated first with 300 c.c. of absolute alcohol with frequent shaking. After three days the clear liquid was decanted as much as possible through a dry filter, and the residue again macerated with 200 c.c. of fresh alcohol. This was decanted at the end of three days and the residue thrown on the filter and washed with enough alcohol to take the place of that retained by the marc, bringing the filtrate up to 500 c.c. Two determinations of total extract as before gave

A	B	Average.
3.80	3.70	3.75 per cent.

It was not found practicable to completely extract sage with alcohol by cold maceration, and subsequent determinations of other extracts are made on residues from Soxhlet extraction by alcohol.

Estimation by Difference: The residue of 3 gms. of sage after ex-

traction by ether was brought to constant weight at 100° and then extracted with absolute alcohol for 40 hours, when the residue was again dried at 100° to constant weight and the loss noted.

Weight residue before.....	2.6532
“ “ after	2.3030
Weight alcoholic extract.....	0.3503
Per cent. “ “	11.68

The alcoholic solution had an intense bright green color, showing a blood-red color by transmitted light, due to chlorophyll. Upon evaporation of the alcohol it left a green, tenacious residue having a sweetish, followed by a bitter taste. It contained no alkaloids or glucosides. The following determinations were made :

Tannin : A qualitative determination of tannin was first made in the alcoholic extract, the amount was afterwards determined in a water-solution of the fresh sample. Sage was thus shown to contain an iron-greening tannin to the amount of 4.0 per cent.¹

Dextrose : See pp. 14-15.

Organic Acids : 300 c.c. of the alcoholic extract of 20 gms. of sage was evaporated to dryness on a water-bath and the residue then extracted with a measured quantity of water. The water solution was treated with solution of lead acetate, when a slight yellow precipitate resulted. It did not become granular on standing, and its amount was too small for further work. A detailed examination for acids, especially malic acid, appears later. (See pp. 18-20.)

EXTRACTION WITH WATER.

Direct Estimation : Two residues (A) from 10 gms., and (B) from 3 gms. of sage after extraction with previous solvents were macerated respectively with 100 c.c. and with 30 c.c. of water, with frequent shaking, during 24 hours. They were then filtered and the residues washed with water till the washings came through clear. (A) was made up to 500 c.c., (B) to 250 c.c. ; and 50 c.c. of each was evaporated to dryness on a water-bath and then dried at 110° to constant weight. The residues were then incinerated and the ash deducted.

¹ For details of the examination for tannin see pp. 15-18.

	A	B
Weight residue.....	0.173	0.0850
Weight ash.....	0.045	0.0221
Water extract	0.128	0.0629
Per cent. water extract.....	12.8	10.48
Average	11.64 per cent.	

It was found almost impossible to bring the water extracts to constant weight without change in their composition which accounts for the variation in the weighings. The more reliable index of the amount extracted by water is that determined by difference.

Estimation by Difference: Two residues, each representing 3 gms. of original sample, after extraction by previous solvents, were extracted with water as above for 24 hrs., and the resulting residues thoroughly dried first in the air and then in a water-oven at 100° to constant weight. The loss represents the total extract plus the ash which latter was calculated from the determination previously made in the extract obtained direct, and deducted.

Weight residue before	1.8940	1.8677
Weight residue after	1.3672	1.3408
Loss	0.5268	0.5269
Ash (calculated)	0.1369	0.1369
Weight water extract	0.3898	0.3899
Per cent. water extract.....	12.99	12.99

“Mucilaginous substances, etc.” (Dragendorff): 50 c.c. of the water solution from (A) was treated with 100 c.c. of absolute alcohol. A brown, flocculent precipitate separated which was filtered after 24 hours on a tared filter and after washing with 66 per cent. alcohol, ^gdried at 100° and weighed. It was then incinerated and the ash deducted.

Weight precipitated.....	0.0454
Weight ash.....	0.0144
Weight mucilaginous and other substances ppt'd by alcohol	0.031
Per cent.....	3.1

For dextrose see pp. 14-15; for organic acids see pp. 18-20.

EXTRACTION WITH SODIUM HYDROXIDE.

Two residues from extractions by previous solvents (A) of 3 gms. and (B) of 5 gms. of sage originally, were macerated with ten times

these numbers of cubic centimeters respectively of 0.15-per-cent. solution of sodium hydroxide, with frequent shaking during 24 hours, after which time the residues were filtered out, well washed with water (till the washings were colorless), and dried to constant weight at 100°.

	A	B
Weight residue before.....	1.3672	2.5425
“ “ after.....	1.2427	2.3305
Weight NaOH extract.....	0.1245	0.2145
Per cent. “ “	4.15	4.29
Average NaOH extract	4.22 per cent.	

The soda solution was colored a dark reddish brown, and contained the usual mucilaginous and decomposition products.

EXTRACTION WITH DILUTE ACID.

Two residues (A) of 3 gms. and (B) of 5 gms. of sage previously extracted as described were macerated with 30 c.c. and 50 c.c. of a solution of hydrochloric acid of 1.13 per cent. strength. After 24 hours the residues were filtered out, and washed with water till the washings no longer reacted acid to litmus. The residues were then dried at 100° to constant weight.

	A	B
Weight residue before.....	2.3305	1.2427
Weight residue after.....	2.1800	1.1463
Loss.....	0.1505	0.0964
Per cent. loss.....	3.01	3.21
Average	3.11 per cent.	

The acid solution was colorless.

For a determination of sugar see pp. 14-15.

LIGNIN, CELLULOSE, ETC.

The lignin, cellulose, etc., were determined according to the method of Cross and Bevan.¹

A residue from extraction by previous solvents weighing 2.1717 gms. was boiled for 30 minutes with 55 c.c. of a 1-per-cent. NaOH solution, filtered hot and washed with water till the washings were colorless, and free from alkali. The residue was too compact to allow of passing in chlorine gas directly, and was therefore suspended

¹ *Journ. Chem. Soc.*, 55, p. 199.

in as little water as possible and chlorine gas passed through it with occasional stirring for an hour. The color of the residue changed rapidly from a brown to a light yellow. It was filtered out and washed with water till the washings were no longer acid, then transferred to a beaker containing 40 c.c. of a 2-per-cent. sodium sulphite solution and 8 c.c. of a 1-per-cent. NaOH solution, and the whole boiled for five minutes and filtered. The residue was well washed with water to remove salts, dried in the air, and finally at 100° to constant weight. The loss was noted as lignin, incrusting and cuticular substances, the residue was calculated as cellulose.

Weight residue before.....	2.1717
Weight residue after	0.7365
Weight lignin, etc.....	1.4352
Per cent. lignin, etc.....	28.7

The residue of cellulose was then incinerated and the ash deducted (such having once been determined).

Weight residue	0.7365
Weight ash.....	0.0980
Weight cellulose (direct).....	0.6385
Per cent. cellulose (direct).....	12.77

TABLE OF TOTAL EXTRACTS.

	By Difference, Hot.	Direct.	
		Hot.	Cold.
Moisture	8.54	8.54	8.54
Ash.....	10.91	10.91	10.91
Petroleum-ether extract.....	4.99	4.47	4.93
Ethyl-ether extract	4.18	4.18	4.93
Alcohol extract.....	11.68	12.88	3.75 ¹
Water extract.....	12.99	11.64	11.64
Sodium-hydroxide extract.....	4.22	4.22	4.22
Hydrochloric-acid extract	3.11	3.11	3.11
Lignin.....	28.70	28.70	28.70
Cellulose.....	10.68	12.77	12.77
Total	100.00	101.42	

EXAMINATION FOR SPECIAL CONSTITUENTS.

Total Nitrogen and Proteid Matter: (1) Three portions of sage of 3 gms. each were treated according to the Gunning modification of Kjeldahl's method.

¹ All subsequent extractions were made upon residues after extraction by hot alcohol, since cold extraction was incomplete.

	A	B	C
Per cent. total nitrogen.....	2.27	2.25	2.29
Average.....	2.27		
Per cent. protein ($N \times 6.25$).....	14.19		

(2) Five grams of sage was macerated in a 500-c.c. flask with about 450 c.c. of cold water for 24 hours. The volume was then made up to 500 c.c. and the nitrogen determined in 100 c.c. of the clear solution.

Per cent. nitrogen in water solution.....	0.71
Per cent. protein in water solution.....	4.44

(3) The residue of 5 gms. of sage after extraction with water was macerated for 24 hours with a 0.15-per-cent. solution of sodium hydroxide. The solution was made up to 500 c.c. and the nitrogen determined in 100 c.c. as above.

Per cent. nitrogen in alkali extract.....	0.56
“ “ protein “ “ “	3.50

(4) The residue of 5 gms. of sage from the extraction with soda was heated with 10 gms. potassium sulphate and 25 c.c. sulphuric acid till all of the organic matter was destroyed, when the nitrogen was determined as usual.

Per cent. nitrogen in residue.....	1.02
“ “ protein “ “	6.37

These results are interesting in that over two-fifths of the proteid matter of sage is shown to be insoluble in water and cold, dilute, alkali.

Resin: The ethereal solution of 50 gms. of sage was freed from solvent, extracted with water, and finally dissolved in alcohol as described (p. 8). The alcohol was evaporated off on a water-bath leaving a glistening, dark green mass, which was easily reduced to an amorphous pea-green powder, soluble in chloroform, but insoluble in benzol. It was insoluble in cold caustic alkali, but dissolved in a boiling solution of one per cent. NaOH, and more readily in hot alcoholic potash, forming brown solutions from which it was precipitated on acidifying with sulphuric acid as a green flocculent mass. It proved to be an acid resin, and amounted to 3.57 per cent. of dry sage.

Carbohydrates: The alcoholic and the water extracts showed a reducing power, due in part to the tannin present. The reducing

sugar appeared to be dextrose or invert sugar, since the usual qualitative tests¹ failed to show mannose or galactose, or more than traces of pentose sugars. Owing to the presence of tannin and other soluble constituents, the direct determination of reducing sugars was not considered satisfactory.

A portion of sage was extracted with water, the filtered liquid shaken with hide powder to remove the tannin, and a portion of the de-tannated solution inverted. The reducing power of the solution thus obtained representing the total water-soluble carbohydrates was equivalent to 8 per cent. dextrose, or 7.20 per cent. dextrin. No starch could be found either in a hot water extract of sage direct, or upon treatment of sage itself with iodine under the microscope.

The residue from the water extraction was boiled three hours with 3-per-cent. hydrochloric acid using a reflux condenser, the solution neutralized and made up to volume, and the reducing power determined as usual. This calculated as dextrose was equivalent to 10.08 per cent. as 9.72 per cent. of anhydride $C_6H_{10}O_5$. Calculated to pentosans the result would be slightly lower.

The hydrochloric acid extract was tested qualitatively like the water extract and showed no indication of mannose, or of more than traces of galactose, but did give a strong reaction for pentoses, indicating that the hemicellulose of sage is composed largely of pentosans, but contains little if any mannan or galactan.

Tannin: A direct aqueous extract of the sample gave evidences of the presence of an iron-greening tannin, for the detection and estimation of which the following methods were employed:

1. Qualitative: In order to have the solution as free as possible from resin, fats, coloring and other foreign matter, the qualitative tests for tannin were made upon the alcoholic extract gotten in course of the usual analysis after previous extraction by ether and petroleum ether. For this purpose 300 cc. of the alcoholic solution by cold extraction (see p. 9) of 50 gms. of sage originally was used. To guard against decomposition that might result from evaporation of the solvent in the air, the solution was transferred to a distilling

¹ The tests used were: for mannose, attempt to form the hydrazone (E. Fischer, Ber. 21, p. 1805) for galactose, oxidation with nitric acid and examination for mucic acid (Kent & Tollens, A. Ch. 227, 228); for pentoses, distillation with hydrochloric acid and test for furfural by aniline acetate.

flask, and two-thirds of the alcohol distilled off under diminished pressure. The residue, a dark green, syrupy liquid was then transferred to a shallow dish and dried over sulphuric acid in a desiccator in which a partial vacuum was maintained. The process of evaporation was very slow, requiring over two weeks. At the end of that time there remained a dark green tenacious residue. It had a pleasant aromatic odor, and a sweet, followed by a bitter taste. It was repeatedly treated with water giving a cloudy yellowish solution, which was not easily filtered clear. The aqueous solution was employed in the tests for tannin which follow. The reagents used were dilute; and the results of each test were compared with a solution of pure tannin. The appended table shows the reactions of the sample compared with those given by a typical iron-greening tannin.

Reagent.	Sample.	Typical Iron-greening Tannin.
Ferric chloride.	green color; green ppt.	do to green-black ppt.
Ferrous sulphate.	green color; sl. green ppt.	do.
Lead acetate.	yellow cloud.	flesh-col. to yellow-white ppt.
Lime water.	sl. yellow to white ppt.	yellow, red-brown ppt.
Bromine water.	sl. yellow cloud.	yellow-brown ppt.
Pot. dichromate.	brown ppt.	brown ppt.
Cupric acetate.	sl. green to black ppt.	brown, green, black ppt.
Uranium acetate.	sl. red color; dark brown ppt.	red-brown ppt.
Pot. ferrocyanide and ammonia.	red to brown coloration.	red, changing to brown color.
Pot. iodide, iodine and ammonia.	sl. brown ppt.	red, changing to brown color.

Considering the wide variation in the behavior of tannins toward reagents, these results point quite conclusively to the presence of a so-called iron-greening or pyrocatechol tannin, presumably in small quantity. To determine its amount the following method was employed:

2. Quantitative: To determine the tannin quantitatively, the method of Löwenthal² as modified by Schroeder was used.³

The procedure was that employed by Proctor.⁴ An aqueous extract of the sample was titrated with permanganate, using a measured amount of indigo-carmin as an indicator. The amount of

¹ See Trimble: *The Tannins*, II., p. 91.—Proctor: *Leather Industries*, pp. 68–89.

² *Jour. f. Prak. Chem.*, III, p. 150. 1860.

³ Loc. cit., *Ber. der Comm. zur Feststellung einer einheitlichen Methode der Gerbstoff-bestimmung*, Cassel. 1885.

⁴ *Ibid.*, loc. cit.

permanganate necessary to turn the indicator was previously determined, and the value of the permanganate in terms of tannin gotten by titration against a solution of pure tannin. The usual correction for the presence of gallic acid, etc., in prepared tannin was made by multiplying by 1.05. The amount of moisture in the standard tannin was also determined and a proper correction made.

Since other substances besides tannin might be present that would reduce permanganate, the tannin was later removed by gelatine, and the filtrate again titrated, the difference being calculated to tannin. The solutions used were: Potassium permanganate 0.5 gm. per liter, indigo-carmin 5 gms. and concentrated sulphuric acid 50 gms. per liter; pure (?) tannin 3 gms. per liter.

Five gms. of sage in a fine powder was first macerated for one-half hour with 300 c.c. of water in a 500-c.c. flask, then boiled for one-half hour, the whole made up to the mark, and, after shaking, filtered.

Three grams of pure tannin was heated in a water-oven for two hours.

Weight before.....	2.0000
Weight after.....	1.8129
Loss	0.1871
Per cent. moisture.....	9.35

Three gms. tannin used therefore correspond to 2.7195 gms. dry tannin, which multiplied by the factor 1.05 gives 2.8554 gms. Each cubic centimeter of the standard tannin solution therefore = 0.00285 gm. tannin.

25 c.c. indigo sol. and 5 c.c. tannin sol. required	52.70 c.c. permanganate
25 c.c. indigo solution required.....	29.93 c.c. “
Therefore 5 c.c. tannin solution required	22.77 c.c. “

Each c.c. permanganate = 0.000626 gm. tannin.

25 c.c. indigo solution and 5 c.c. sample required	34.10 c.c. permanganate
25 c.c. indigo solution required.....	29.93 c.c. “
Therefore 5 c.c. sample required.....	4.17 c.c. “

The tannin was then removed as follows and aliquot parts of the filtrate titrated as before. To 50 c.c. of the original water extract, 28.6 c.c. of a solution of 2 gms. of gelatine in 100 c.c. of water was added, and the whole carefully shaken. To the mixture was then added small portions of salt until no more was dissolved. Ten cubic

centimeters of dilute sulphuric acid (1-9) and 20-30 gms. of kaolin was then added to hasten filtration, and after shaking the whole was filtered, being previously made up to 100 c.c.

10 c.c. of this solution = 5 c.c. of original solution.

25 c.c. indigo solution and 10 c.c. filtered solution required 30.9 c.c. permanganate.

25 c.c. indigo solution required 29.93 c.c. permanganate.

Therefore 10 c.c. of filtered solution (= 5 c.c. original solution) required 0.97 c.c. permanganate.

5 c.c. original solution required 4.17 c.c. permanganate.

5 c.c. de-tannated solution required 0.99 c.c. permanganate.

Therefore 5 c.c. original solution contained tannin requiring 3.2 c.c. permanganate.

1 c.c. permanganate = 0.000626 gm. tannin.

3.2 c.c. permanganate = 0.002 gm. tannin.

Per cent. tannin = 4.0.

Examination for Acids: The amounts of material used in course of the usual analysis proved too small to allow of proper tests, and the examination for organic acids was made direct upon water extracts of sage. The first examination was made upon a portion of dry sage of the same lot previously used; but as it was thought that there was a possibility of some change between the dry and the fresh plant, a second examination was made upon a portion of freshly-picked sage. As the methods employed were the same in both cases, and the results practically identical, the details of the examination of the fresh sage only are given.

The absence of gallic, benzoic and salicylic acids has already been shown (p. 8). Ilisch and Riga in 1810,¹ working upon a large quantity of fresh sage reported free malic acid as a constituent upon what would appear to be insufficient evidence, their conclusion apparently being based entirely upon physical properties of an impure acid residue. I have repeated their work, using smaller quantities of material, and have succeeded in obtaining such a residue as described in an amount, however, too small for the purification necessary for a combustion. Though malic acid is included in various works of reference as a constituent of sage, I have been unable to find any further authority upon which such statements may be based beyond that given.

¹ Trommsdorff's Jour. der Pharmacie, 20, II, pp. 7-16.

At the time of this analysis (February) fresh sage was not procurable in New York, and the sample for this examination was obtained from St. Augustine, Florida. It had, however, not been picked longer than ten days.

About 30 gms. of fresh sage was picked free from foreign substances, and bruised in a mortar. It was not possible to press out the juice direct using a screw-press, and the drug was first steeped in cold water and pressed. This was repeated several times; solution (A). The residue was then similarly extracted and pressed using warm water; solution (B). The residue remaining was then boiled with water, filtered and the residue again pressed; solution (C). All of these extracts were brown in color. They did not react acid to litmus. Hence free malic acid was absent.

(A) The method here used is a combination of that of Liebig,¹ and of Winckler.²

To the aqueous extract, an excess of a neutral solution of lead acetate was added. The brownish green slimy precipitate that resulted, carried down practically all of the coloring matter and tannin also. It was filtered out after standing over night, and washed till the washings were free from lead. The precipitate while still moist was transferred to a small beaker, suspended in water, and a stream of hydrogen sulphide run in. The lead was thus thrown out as a sulphide which carried with it the greater part of the coloring matter. The precipitated sulphide was filtered out, and the filtrate and washings evaporated first on a sand-bath to one-third of the volume and finally on a water-bath. There remained a small amount of a brownish liquid containing a few granules of lead sulphide. It was re-dissolved, filtered and again evaporated. A slight syrupy residue remained. An attempt was made to purify this residue and get it to crystallize. For this purpose bone-black was freed from carbonates by boiling with dilute nitric acid, then repeatedly with water to remove the excess of acid and finally dried in an air-bath. The syrupy residue was then dissolved in water and boiled with the bone-black thus prepared. It was finally filtered and the filtrate evaporated on a water-bath as before. There remained a slight amount of a colorless syrup, which reacted acid to litmus.

¹ *Pogg.*, 18, 357; *do.*, 28, 195.—*Ann. Pharm.*, 26, 166.

² *Jahresb. pr. Pharm.*, 1, 13.

This, however, cannot be taken as an indication of the presence of an acid originally in the sage, for it might easily be due to traces of acetic acid remaining from the precipitation by lead acetate.

(B) The second aqueous extract was treated in the same way as above with a similar result.

(C) The extract was divided into two parts for examination, particularly for malic acid.

1. The water solution was precipitated by lead acetate as before, the lead precipitate filtered out and well washed with cold water, and finally dried to a glistening, brittle, green mass, which was powdered. If malate of lead were present, it should be dissolved out by boiling water.¹ The powdered precipitate was then boiled with small amounts of water and each time filtered hot. The filtrates on cooling deposited small amounts of a brown, amorphous powder, containing nothing crystalline, and consisting probably largely of coloring matter, tannin, etc.

2. The second portion was boiled with milk of lime for half an hour, when a brown powder separated, which was filtered out and washed. After drying the whole was thrown into a boiling mixture of 1 part of nitric acid and 10 parts of water,² and boiled for half an hour. On cooling, no acid malate of lime was thrown down.

The above acid residues titrated with soda showed that the possible quantity of organic acids in sage cannot be greater than a fraction of a per cent.

THE DETERMINATION OF THE OIL OF SAGE.

There is at present no uniform method in use for the estimation of essential oils in plants, or in mixtures of essential oils with fixed oils, resins, extractive matters and the like. Various methods are employed as seem best fitted to the particular oil to be determined, and the nature of the other substances occurring with it. In most cases, these determinations are carried on without corroboratory checks by other methods, thus giving no means of comparison as to the accuracy of the particular method employed.

Because of the wide variation in the composition of essential oils, and the lack of uniformity in their behavior toward reagents, to-

¹ Vaquelin: *Ann. Chim.*, 34, 127.

² *Liebig's Annalen*, 38, 259.

gether with the comparative inertness of some of their most commonly occurring constituents purely chemical methods of determination are not usually applicable. Recourse is usually had to differences in the physical properties of essential oils from those substances occurring with them, especially differences in solubility and ease of volatilization, the latter being most commonly utilized.

In the analysis of plants and other raw products the essential oil is estimated in an extract of the same in (1) petroleum ether, (2) ethyl ether, (3) benzene or (4) carbon bisulphide. These solvents dissolve also as a rule any fixed oil present, together with waxes, certain organic acids, some chlorophyll, and possibly also more or less of alkaloids and resin, the latter being brought into solution by the presence of the oils.

The usual methods for the determination of volatile oil may be divided into two general classes: first, those in which the oil is estimated directly from the plant; and second those in which the oil is estimated in an extract from the plant.

1. DETERMINATION OF THE OIL DIRECT FROM SAMPLE: These methods consist in determining the total matter volatile at temperatures of from 100° to 110° , then determining the moisture and calculating the volatile oil by difference.

In this way Thresh¹ determined the volatile oil in ginger. McGill employed a similar method in the analysis of cloves for the determination of volatile oil.² Moisture was determined by drying the finely powdered sample in a vacuum desiccator over sulphuric acid for 30–60 hours. The total volatile matter was then determined by heating a fresh sample in a water-oven for 24 hours, and the volatile oil determined by difference. A similar determination of the total volatile matter after first macerating the finely powdered substance (cloves) with petroleum ether and then drying to constant weight at 100° , and subtracting the moisture gave results from 2–5 per cent. higher in volatile oil. The two chief objections attached to this method are first, the impossibility of accurately determining the moisture apart from the volatile oil; and second, the error due to oil changed or unvolatilized during heating for the determination of total volatile matter. The following experiments

¹ *Pharm. Jour. & Trans.* (3), X, 191.

² Bull. 75 Internal Revenue Dept. Canada, October 1900, p. 7.

were undertaken with a view of determining the error possible because of the first difficulty; the second cause of error applies also to the other methods used and is taken up later.

The methods for determining moisture in the presence of a volatile oil in plants are at best quite arbitrary. Generally the finely powdered substance is heated in a water-oven at 100° for 2–3 hours, and the oil then separately determined in the petroleum ether extract. A determination of the moisture and of essential oil in sage by this means gave (see p. 4) :

	1	2	3
Moisture, per cent.....	8.70	8.65	8.35
Moisture (average)	8.54 per cent.		
Volatile oil.....	0.96	“	“
Total volatile matter.....	9.50	“	“

McGill proposed¹ to determine the moisture apart from the oil, as follows: a sample of the substance under examination is spread out in a thin layer, and dried over sulphuric acid *in vacuo*, until the acid first becomes tinged, when the residue is weighed and the loss calculated as moisture. McGill's method is based upon the assumption that no oil is volatilized under these conditions, until all of the moisture has been given off and absorbed, this point being indicated by the acid becoming discolored by the volatilization and subsequent carbonization of the first trace of oil vapor.

A trial of this method was made as follows: Two portions of powdered sage (A) 5 gms. and (B) 3 gms. were spread out in thin layers and dried over sulphuric acid in a desiccator in which a partial vacuum was maintained. The acid became tinged after about 30 hours, when the residues were weighed.

(A) 5 gms. lost.....	0.2857 gm. = 5.71 per cent.
(B) 3 gms. lost.....	0.1691 gm. = 5.64 “
Average loss	5.67 “
Volatile oil (see p. 29).....	0.96 “
Total volatile matter.....	6.63 “

The total volatile matter, taking the sum of the moisture as thus determined and the volatile oil as afterwards determined in the petroleum-ether extract = 6.63 per cent. The total volatile matter, taking the moisture as determined by heating the sample at 100°

¹ Ibid., loc. cit.

and the oil as above = 9.50 per cent. A deficiency according to McGill's method is thus shown of 2.87 per cent. This deficiency is calculated from the total volatile matter made up of the sum of the moisture determined by heating at 100° and the oil as determined in the petroleum-ether extract as stated. If the total volatile matter were determined by heating the sample in a water-oven for 24 hours as suggested by McGill the deficiency would be even greater.

2. INDIRECT DETERMINATION OF THE OIL IN AN EXTRACT PREPARED FROM THE SAMPLE: The indirect methods for the determination of volatile oil in extracts prepared from plants, etc., can be divided into two classes: first, those depending upon the determination of the coefficient of evaporation of the oil at ordinary temperatures; and second, those depending upon the determination of the volatile oil by heating at higher temperatures.

Of the first class, the method of Osse¹ is the only one used. It consists in determining the so-called coefficient of evaporation of the residue left after driving off the solvent, and from the information thus obtained, calculating the amount of volatile oil present. Using pure oil, Osse obtained fairly accurate results, but the method is not adapted to mixtures containing also resins, fats, etc., and has the disadvantage of being open to errors due to slight differences in weighing.

The method usually adopted in the determination of volatile oil in plant analysis² consists in extracting the material with a suitable solvent, evaporating off the solvent, weighing the residue, which is then heated at temperatures varying from 100° to 120° and again weighed, the loss being noted as volatile oil.

This method is open to several errors as will be shown, as follows:

- 1st. Error due to loss of oil during evaporation of the solvent.
- 2d. Error due to incomplete removal of the solvent.
- 3d. Error due to oil not volatilized during the heating.
- 4th. Error due to the influence of foreign substances present with the oil.

It has been found possible to determine and make a correction for the third and fourth errors. Those due to the first and second can

¹ *Archiv d. Pharm.*, 3, vii, 104.

² See Dragendorff's *Pflanzenanalyse*, pp. 22, etc.—Parsons, *Chem. News*, 41, pp. 256, 257.

only be regulated by special treatment according to the nature of the material dealt with.

The first would tend toward low results, the second high, the third low and the fourth, results that would vary with the nature of the material.

1. Error Due to Loss of Oil During the Evaporation of the Solvent :

The only means at our disposal for determining when all of the solvent has been dissipated, lies in noting when it is no longer to be detected by its odor. In the course of an ordinary plant analysis where the amount of oil present is small, this point can be noted with considerable accuracy. In cases where strong-smelling oils are present, or with comparatively pure solutions of essential oils in petroleum ether, such is determined with considerable difficulty.

3.3078 gms. sage oil was dissolved in 200 c.c. of petroleum ether, 5 aliquot portions of 25 c.c. each taken out into lead capsules, and the same exposed to the air at the ordinary temperature till the odor of petroleum ether was no longer apparent, when the capsules were covered and weighed.¹

	A	B	C	D	E
Weight oil theory.....	0.41348	0.41348	0.41348	0.41348	0.41348
Weight oil found.....	0.26350	0.33060	0.33130	0.37150	0.33400
Weight oil lost.....	0.14998	0.08288	0.0822	0.04198	0.07948
Per cent. loss.....	36.27	20.12	19.89	10.37	19.22

The results show the wide range of error possible when relying on odor alone to determine when all of the solvent has been dissipated. The conditions here are unusual ; with extracts given in the course of the ordinary plant analysis, where the amount of oil is small, and the residue has a firmer consistency, the point of total evaporation of the solvent is much more accurately determined.

The only object of weighing the residue after evaporation of the solvent is to determine the total extracted matter upon which to calculate the further loss on heating due to volatile oil. Where such is impossible on account of the difficulty of determining when all of the solvent has been evaporated, the total extract can be determined by difference as described on pages 3-4 ; then the resi-

¹The evaporations were made either in small glass dishes with vertical sides and ground edges, or in lead capsules. By the use of the latter, creeping was lessened. Both were weighed with ground-glass covers.

due of the extract after evaporation of the solvent can be directly heated, the difference between the total extract as found by difference and the residue after heating at 110° being calculated to volatile oil.

2. *Error Due to the Incomplete Removal of the Solvent:* This error applies in the same manner as the first, leading, however, to high results. It is especially noticeable in the case of extracts of a sticky consistency, where all of the solvent is not driven off by simply drying in the air, as is usually done in plant analysis, thus making possible, as will be seen, an error of as much as several per cent. in the total extract and a corresponding error in the volatile oil.

The petroleum-ether extract of 3 gms. of sage was made up to 100 c.c. Four portions of 20 c.c. each were exposed to the air in tared glass dishes till the odor of petroleum ether was no longer apparent. The residues were then weighed, and after carefully heating in a water-oven for ten minutes weighed again. Each portion corresponded to 0.6 gm. of sage.

	A	B
Weight residue air-dried.....	0.0575	0.0545
Per cent. " " "	9.58	9.08
Weight after heating.....	0.0259	0.0259
Per cent. " "	4.32	4.32

A similar extract of 5 gms. of sage was made up to 100 c.c. and two portions of 20 c.c. similarly dried in the air and weighed; then heated for 10 minutes at $60-70^{\circ}$ and again weighed.

Each portion corresponded to 1 gm. sage.

Weight residue air-dried.....	0.1287	0.1264
Per cent. " " "	12.87	12.64
Weight after heating.....	0.0449	0.0444
Per cent. " "	4.49	4.44

It will be noticed that weighing the air-dried extracts in the above determinations gave results varying by something over 3 per cent., due to some of the solvent having been retained by the residue. A number of determinations at various times have shown like variations. The residues in each case after but a few minutes' heating at a temperature somewhat above the boiling point of petroleum ether, gave very constant results, viz.:

4.32 4.32 4.49 4.44 per cent.

which agree tolerably well with the determination of the total extract by difference.

Since an error in the determination of the total extract results in a corresponding error in the determination of volatile oil the conclusions to be drawn from the above are (1) that the weighing of the air-dried extract gives high results, which (2) may be obviated by drying for a few minutes at a temperature somewhat above the boiling point of the solvent. While there may be a slight loss of volatile oil during this short preliminary heating, the error would be extremely small as compared with that resulting from the weighing of the air-dry residues.

3. *Error Due to Unvolatilized Oil*: All essential oils suffer more or less decomposition on heating with the formation of non-volatile products, thus resulting in low figures. This is particularly true of oils containing much pinene, such as the oils of sage, thyme, parsley and lemon, in which the pinene might be converted into terephthalic, terpenylic and terebic acids by the combined action of the air and heat.

Five portions of sage oil were weighed out by difference into glass dishes and heated for 4 hours at 110° in an air-bath.

	A	B	C	D	E
Weight oil taken.....	0.076	0.0723	0.0758	0.0764	0.0750
Weight residue.....	0.0014	0.0012	0.0015	0.0012	0.0015
Per cent. oil unvolatilized	1.84	1.65	1.98	1.57	2.00
Average.....	1.81 per cent.				

If the error due to non-volatilized oil were constant, a correction might be made in the determination of the oil in an extract, from that given by heating the pure oil. The amount unvolatilized was, however, found to vary with the conditions under which the determinations were made and the nature of admixed substances.

Different results were given when the oil had been dissolved in petroleum ether previous to heating from those given by heating the oil direct.

2.9098 gm. oil sage was dissolved in petroleum ether, and the solution made up to 100 c.c. Ten portions of 10 c.c. each were freed from solvent in the air, then heated 4 hours in a calcium chloride bath at 110° and the residues weighed.

	A	B	C	D	E
Weight oil taken	0.29098	0.29098	0.29098	0.29098	0.29098
Weight residue	0.00568	0.00633	0.00660	0.0059	0.0057
Per cent. residue	1.933	2.175	2.268	2.028	1.993

	F	G	H	I	J
Weight oil taken	0.29098	0.29098	0.29098	0.29098	0.29098
Weight residue	0.00775	0.0079	0.00575	0.0053	0.0043
Per cent. residue	2.663	2.715	1.976	1.821	1.478

Average for oil unvolatilized at 100° = 2.175 per cent.

4. *Error Due to the Influence of Foreign Matters Present with the Oil:* Osse¹ experimenting upon the action of fixed oil in preventing the volatilization of the essential oil, or by its oxidation, leading to low results, found that a close approximation to the truth might be arrived at by deducting 0.09 to 0.1 per cent. from the weight after heating at 110°. He further concluded that no appreciable error resulted from oxidation of the fixed oil during the evaporation of the petroleum ether, as the presence of the latter, even in small quantities, prevent such change. Dragendorff² obtained similar results with admixtures of cocoa butter, and in addition found that resin could be completely freed from essential oil at 100–110°; and it was only in the case of oils prone to oxidation that the residual resin was somewhat heavier than was expected.

In the method suggested later it is immaterial what influence foreign substances have on the volatilization of the essential oil, and further experiments in this direction were therefore not made.

The first two errors, being errors of manipulation can, only be obviated by a study of the best conditions under which to determine the oil in each special case. To arrive at a proper correction for the second two, I have used the following method.

The material, usually 3–5 gms. is extracted with petroleum ether as usual, and the solution made up to a convenient volume, preferably 100 c.c. Several aliquot parts of this are taken out, by means of a pipette into small covered glass dishes and to one or more of these is added a known amount of the pure oil of the kind occurring in the material in which the oil is to be determined. The extracts are then freed as completely as possible from the solvent in the air, and the last traces removed by heating for ten minutes in a

¹ *Archiv d. Pharm.* [3], VII, 104.

² *Pflanzenanalyse*; p. 20, etc.

water-oven at 60–80° (if petroleum ether has been used), when the residues are weighed and an average taken for total extract. It is not necessary to weigh the dishes to which oil has been added, at this time. All of the dishes are then heated at 110° for 4 hours best in a calcium chloride bath, which can be easily regulated, when they are cooled, covered and weighed. The difference between the average weight of the dishes to which no oil has been added, before and after heating, represents the apparent amount of volatile oil present in the sample. Since aliquot parts of the original solution were used, the difference between the average weight after heating of the dishes to which no oil was added and the average weight of those to which an amount of oil was added, will show the amount of the added oil that remains unvolatilized under the conditions of each determination. The error due to unvolatilized oil is thus determined and a proper correction made. If necessary, the total extract may be determined by difference, and the calculation made from the figure thus obtained without weighing for total extract direct. The following determination of the volatile oil in sage was made by this method:

5 gms. of sage was extracted with petroleum ether as usual, the solution made up to 100 c.c., and 4 portions of 20 c.c. each taken out into tared dishes (A, B, C and D). To (C) was added 0.24 gm. pure oil sage; to (D) 0.1785 gm. The four were then exposed to the air till most of the solvent had evaporated, and then heated in a water-oven at 60–70° for ten minutes. (A) and (B) were then weighed for total extract. Each portion represented 1 gm. sage.

	A	B
Weight total extract.....	0.0449	0.0444
Per cent. total extract.....	4.49	4.44

All four dishes were then heated at 110° for four hours, and again weighed.

	A	B
Weight before.....	0.0449	0.0444
Weight after.....	0.0354	0.0354
Average.....	0.0354	
Loss.....	0.0095	0.0090
Per cent. loss	0.95	0.90
Average.....	0.925 per cent.	
Weight after.....(C)	0.0438	(D) 0.0425

Average weight after of (A) and (B).....	0.0354	0.0354
Weight oil unvolatilized.....	0.0084	0.0071
Per cent. oil unvolatilized.....	3.50	3.98
Average.....	3.73 per cent.	

Thus the loss at 110° as determined in (A) and (B) represents $100 - 3.73$ per cent. = 96.27 per cent. of the actual oil present. The essential oil in sage is then determined from the proportion :

$$0.925 : x :: 96.27 : 100.$$

$$\text{Essential oil} = 0.96 \text{ per cent.}$$

SUMMARY AND CONCLUSIONS.

1. The proximate composition of sage was determined both by the method of Dragendorff and by hot extraction.

2. The results of cold and hot extractions were found to compare fairly well, except in the case of extraction with alcohol, where cold maceration was found to give very incomplete extraction.

3. An analysis was made in which the various extracts were determined by difference, instead of directly, and was found to give good results.

4. The petroleum-ether extract was shown to contain besides fixed oil, wax and coloring matter, about one per cent. of volatile oil.

The methods for determining volatile oil were studied, the sources of error pointed out, and an improved method proposed.

5. In the ethyl-ether extract 3.5 per cent. of a green resin was found.

6. The presence of about 4 per cent. of an "iron-greening" or pyrocatechol tannin was shown.

7. The total proteid matter amounted to about 14 per cent., of which 4.5 per cent. was soluble in water, 3.5 per cent. in dilute cold alkali; and about 6 per cent. was shown to be insoluble in the above.

8. Starch was found to be absent, and the amount of easily hydrolyzed hemicellulose was small. The latter contained a considerable proportion of pentosans.

9. Free malic acid, as reported by Ilisch and Riga, could not be found.

TABLE OF PRINCIPAL CONSTITUENTS.

Constituent.	Per Cent.
Ash	10.91
Moisture.....	8.54
Essential oil.....	0.96
Fixed oil and wax	3.51
Resin.....	3.57
Tannin.....	4.00
"Mucilaginous substances" (Dragendorff)	3.10
Lignin.....	28.70
Cellulose.....	12.77
Soluble carbohydrates as dextrin	7.20
Hemicellulose yielding reducing sugar.....	9.72
Total proteids ($N \times 6.25$).....	14.19
Proteids soluble in cold water.....	4.44
Proteids soluble in dilute alkali.....	3.50
Proteids insoluble in water and dilute alkali (direct).....	6.37

BIOGRAPHICAL.

Leon Laizer Watters prepared for college at private and public schools.

In June, 1897, he received the degree of Bachelor of Science from the University of Utah.

In October, 1897, he entered Columbia University, receiving the degree of Master of Arts, under the Faculty of Pure Science in June, 1898.

From 1898-1900 he was assistant in chemistry at the College of Physicians and Surgeons of Columbia University.

During 1900-1901 he pursued post-graduate studies in chemistry, under the Faculty of Pure Science of Columbia University, leading to the degree of Doctor of Philosophy.

Previous publications: "The Carbide of Gold" (with J. A. Mathews). *Jour. Am. Chem. Soc.*, Vol. XXII, No. 2, February, 1900, pp. 108-111.

